IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Chatterjee, D.K.

Appl. No. 09/558,421

Filed: April 26, 2000

For: **Mutant DNA Polymerases and Uses Thereof**

Art Unit: 1652

Examiner: Rao, M.

Atty. Docket: 0942.3600003/RWE/BJD

Declaration of Elizabeth Flynn

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Elizabeth Flynn, do hereby declare and say:

1. THAT, I, Elizabeth Flynn, hold the degree of B.S. A recent copy of my Curriculum Vitae, accurately listing my scientific credentials and work experience, is attached hereto as Exhibit A.

2. THAT, since 1994, I have been employed by Life Technologies, Inc. (LTI) (and now Invitrogen Corporation)[1], the assignee of the above-captioned application, in the capacity of Associate Scientist I. *See* Exhibit A.

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[1]Life Technologies, Inc. merged with Invitrogen Corporation on September 12, 2000, with Invitrogen Corporation being the surviving entity.

3. THAT, during my employment by LTI (and now Invitrogen Corporation), I worked under the supervision of Dr. Deb K. Chatterjee on a project involving the cloning, expression, and characterization of wild-type and mutant DNA polymerases.

4. THAT, I have reviewed my laboratory notebooks detailing my work on the project. Based on these laboratory notebook records and my recollection, the following activities involving my work, and relating to the DNA polymerase project, took place during the period from about October 16, 1994, until about September 8, 1995.

On or about December 12, 1994, I conducted an experiment to prepare a purified preparation of the F667Y mutant of *Taq* polymerase. I washed and equilibrated a Super Q 650 column, and added the dialysate from the experiment performed on December 10, 1994, to further purify the F667Y *Taq* polymerase mutant. The fractions from the Q650 column were assayed for polymerase activity, and the active fractions were pooled. This experiment was recorded on pages 102-103 of notebook 3865. A copy thereof is attached as Exhibit 1.

On or about December 13-14, 1994, I continued the experiment to prepare a purified preparation of the *Taq* F667Y mutant of *Taq* polymerase. I optimized the time course and dilutions for the mutant F667Y *Taq*. This experiment was recorded on pages 104-106 of notebook 3865. A copy thereof is attached as Exhibit 2.

On or about March 29, 1995, I began an experiment to purify a new 3' exonuclease mutant of *Tne* polymerase. In this experiment I lysed cells containing the 764-D1-00-1R wild-

type *Tne* polymerase. The lysate was heat treated and PEI and ammonium sulfate precipitations were performed to purify the polymerase. This activity was recorded on page 108 of notebook 3865. A copy thereof is attached as Exhibit 3.

On or about March 30, 1995, I continued the experiment to isolate the 3' exonuclease mutant of *Tne* polymerase. I resuspended the pellet from the ammonium sulfate precipitate obtained on March 29, 1995. The resuspended pellet was dialyzed for 8 hours. Finally, the dialysate was loaded onto a heparin column to purify the polymerase. This experiment was recorded on pages 109-110 of notebook 3865. A copy thereof is attached as Exhibit 4.

On or about March 31, 1995, I continued the experiment to isolate the 3' exonuclease mutant of *Tne* polymerase. I assayed fractions from the heparin column for polymerase The fractions were analyzed by SDS-PAGE, and Bradford protein concentration assays were performed. This experiment was recorded on pages 111-113 of notebook 3865. A copy thereof is attached as Exhibit 5.

On or about April 2, 1995, I performed an experiment to isolate *Tne* wild-type 764-D1-00-1R on a Q650 column. This activity was recorded on page 114 of notebook 3865. A copy thereof is attached as Exhibit 6.

On or about April 3, 1995, I continued the experiment to isolate a *Tne* polymerase mutant by assaying fractions from the Q650 column for polymerase activity. I also assayed the protein

concentration of the fractions. This experiment was recorded on pages 115-117 of notebook 3865. A copy thereof is attached as Exhibit 7.

On or about April 4, 1995, I continued the experiment to isolate a *Tne* polymerase mutant by performing calculations to determine the total activities in the fractions obtained from the Q650 column. These calculations were recorded on page 118 of notebook 3865. A copy thereof is attached as Exhibit 8.

On or about April 5, 1995, I continued the experiment to isolate a *Tne* polymerase mutant by re-assaying the total activities of the purified samples obtained from the Q650 column. I performed calculations to determine the percent yield and analyzed the samples by SDS-PAGE. Fractions were also assayed for RNase activity. This activity was recorded on pages 119-121 of notebook 3865. A copy thereof is attached as Exhibit 9.

On or about April 6, 1995, I continued the experiment to isolate *Tne* polymerase by continuing RNase assays on the newly purified *Tne* mutant. Additionally, I performed exonuclease assays on the *Tne* mutant fractions. This experiment was recorded on pages 122-125 of notebook 3865. A copy thereof is attached as Exhibit 10.

On or about May 17, 1995, Roger Lasken or I conducted a unit assay on the *Tne* polymerase preparation from May 7, 1995. This experimental activity was recorded on page 22 of notebook 3903. A copy thereof is attached as Exhibit L-90.

On or about May 22, 1995, I conducted an experiment to begin purification of the 3' exonuclease mutant of *Tne* polymerase. I lysed host cells transformed with DNA encoding the 3'-5' exonuclease mutant of *Tne* polymerase. Additionally a heparin column was equilibrated. This experimental activity was recorded on page 129 of notebook 3865. A copy thereof is attached as Exhibit 11.

On or about May 25, 1995, I performed an experiment to purify the 3' exonuclease mutant of *Tne* polymerase on a heparin column. Polymerase activities of the heparin column fractions were assayed. The fractions were heat treated. This experimental activity was recorded on pages 130-131 of notebook 3865. A copy thereof is attached as Exhibit 12.

On or about June 13, 1995, I performed an experiment to determine the amount of heat stable polymerase activity in the crude lysate containing *Tne* 3' exonuclease mutants. This experimental activity was recorded on pages 132-133 of notebook 3865. A copy thereof is attached as Exhibit 13.

On or about June 14, 1995, I performed an experiment to reassay the crude lysates obtained from bacteria transformed with the new *Tne* 3' exonuclease mutant and from bacteria transformed with the new FY *Tne* mutant for thermostable polymerase activity. This experimental activity was recorded on pages 134-138 of notebook 3865. A copy thereof is attached as Exhibit 14.

On or about June 15, 1995, I performed an experiment to continue purification of the 3' exonuclease mutants of *Tfl* polymerase. I lysed new batches of cells containing the FY mutant and the 3' exonuclease mutant, performed PEI and ammonium sulfate precipitations on the lysates, and applied the FY and the 3' exonuclease mutant lysates to a heparin column. Fractions obtained from the heparin columns were assayed for polymerase activity. This experimental activity was recorded on pages 139-143 of notebook 3865. A copy thereof is attached as Exhibit 15.

On or about June 16, 1995, I performed an experiment to continue purification of the 3' exonuclease and FY mutants of *Tne* polymerase. On this date I loaded fractions containing the FY *Tne* mutant and the 3' exonuclease *Tne* mutant on a Q650 column. Fractions obtained from the Q650 column were dialyzed overnight. This activity was recorded on pages 146-148 of notebook 3865. A copy thereof is attached as Exhibit 16.

On or about June 19, 1995, I continued work toward purifying the 3' exonuclease mutant of *Tne* polymerase by removing the samples from the experiment performed June 16, 1995 (*see* Exhibit 16), from dialysis. This experimental activity is recorded on page 149 of notebook 3865. A copy thereof is attached at Exhibit 17.

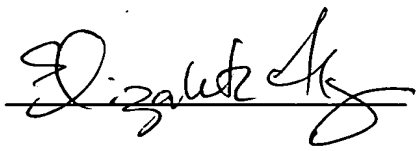
On or about June 27, 1995, I performed an experiment to determine the total activity of the purified fractions of the *Tne* 3' exonuclease mutant. This experimental activity was recorded on pages 150-151 of notebook 3865. A copy thereof is attached as Exhibit 18.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any registration resulting therefrom.

Further, declarant sayeth not.

Date: 11-29-01

Name: Elizabeth Flynn

Signature: 

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Elizabeth Kay Flynn

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Education:

The George Washington University
Institute of Biomedical Sciences
Graduate Program in Genetics (1999-present)
Washington D.C.

University of Maryland Baltimore County
Bachelors of Science in Biochemistry and Molecular Biology (1993)
Baltimore, MD

Skills:

Protein Purification/Analysis
Liquid chromatography- (FPLC, HPLC, TLC)
Analytical and process scale
Native, recombinant, and fusion proteins
Insoluble protein purification and protein refolding
Bacterial, baculovirus, and yeast expression systems
SDS PAGE/Western analysis
Immunoprecipitation
Protein conjugation with reactive dyes
UV/VIS Spectroscopy
Enzymatic Assays

Cloning/Nucleic Acid
Conventional and recombinational cloning (GATEWAY™)
Plasmid construction and purification
Bacterial transformation
Bacterial gene expression systems
Site-directed mutagenesis
PCR
Fluorescent DNA cycle sequencing
Handling of radioactivity

Professional: Life Technologies, a division of Invitrogen Corporation, Rockville, MD
Protein Engineering and Analysis Research and Development
Scientist - Associate Scientist III - (1998 – present)
Research focused on lambda site-specific recombination with regard to recombinational cloning applications.

- Responsible for cloning, expression optimization and purification development of the proteins that make up CLONASE™ enzyme mix, consisting of bacteriophage lambda derived integrase and xicisionase proteins and *E.coli* derived integration host factor.
- Developed a novel robust quantitative radioactive assay for recombinational cloning efficiency used to qualify CLONASE™ enzyme mixes. Responsible for writing production protocols and quality control documents and specifications.
- Isolated a novel stimulatory protein of lambda site-specific recombination.

- Cloned and purified ExoV (RecBCD) from *E.coli* using GATEWAY™ Multisite recombinational cloning. Optimized protein expression in bacterial system and developed the protein purification protocol for manufacturing.
- Other responsibilities include the supervision of two temporary employees, hazardous materials coordinator, and laboratory safety officer.

Associate Scientist II,I – (1994 – 1998)

- Engineered a recombinant protein molecular weight size standard in an unstained and prestained version, the BENCHMARK™ protein Ladders. Involved in cloning, protein expression optimization, development of purification protocols, protein-dye conjugation protocols and final product configuration. Responsible for writing purification protocols and quality control protocols, and determining specifications for manufacturing.
- Contributed to the development of a novel method for the production of insoluble recombinant protein in *E.coli.*
- Involved in developing purification protocols for a variety of proteins including thermostable polymerases, reverse transcriptases, gyrase, and T4 polynucleotide kinase.

University of Maryland Baltimore County
Dr. Richard Karpel - Professor

Research Assistant – (1992-1994)

Research focused on studying biochemical aspects of bacteriophage T4 g32 protein.

- Cloned, expressed, and developed purification protocols for deletion mutants of g32 protein.
- Used oligonucleotide protection proteolysis experiments to further define the DNA binding region of g32 protein.

Publications: Waidner LA, Flynn EK, Wu M, Li X, Karpel RL, Domain effects on the DNA-interactive properties of bacteriophage T4 gene 32 protein. *Journal of Biological Chemistry,* **276**: 2509-2516 (2001)

Wu M, Flynn EK, Karpel RL, Details of the nucleic acid binding site of the T4 gene 32 protein revealed by proteolysis and DNA Tm depression methods, *Journal of Molecular Biology, 286: 1107-1121 (1999).*

Dube S, Flynn EK, Estimating Protein Molecular Weights Using SDS-PAGE, *Focus*, **20(1)**: 24-25 (1998).

Flynn EK, Oberfelder RW, Chatterjee DK, Protein Analysis with the BENCHMARK ™ Protein Ladders, *Focus*, **19(2)**: 33-35 (1997).

Patents pending:

Chatterjee DK, Longo M, Flynn EK
Methods for the Production of Proteins
(filed January 1998)

References: Available upon request.